Microtubular Motors

1858-Pos Board B628
Proton Tunneling Accelerates ATP Hydrolysis in Eg5 Kinesin
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In ATP hydrolysis, a proton from the water nucleophile must be abstracted and transferred in order to create a hydroxide capable of attacking the substrate. Herein, solvent kinetic isotope experiments with Eg5 kinesin show unanticipated accelerated proton transfer involving an active-site water cluster. The positive kinetic isotope effect (KIE) confirms proton abstraction from water commits kinesin to catalysis and its pH-dependence verifies that switch salt-bridge residues direct chemotransduction. Additionally, a classical description for this proton transfer is refuted by the KIE magnitude, temperature-independent Arrhenius pre-exponential factor ratios, and activation energy differences. Taken together, we conclude that the first step in Eg5 catalysis has a tunneling component, a quantum mechanical event by which a particle transfers through a reaction barrier. This first detection of tunneling in an ATPase is of consequence for two reasons. First, proton tunneling is likely widespread in biomolecules, rather than solely a characteristic of metalloenzymes. Second, energy barrier penetration by proton tunneling is an alternate explanation to classical transition-state stabilization theory for the fast reactivities of motor proteins.

1859-Pos Board B629
The Motor Domain Transducer is Key in Kinesin Functional Plasticity
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Kinesin orthologs within the motor protein superfamily recently have been shown to have an unanticipated functional breadth via motor domain-microtubule interactions. This functional range suggests motor domains exhibit functional specialization despite conservation of chemistry, structure, and overall sequence. Herein, our bioinformatic and computational studies uncover a protein sector of the motor domain responsible for tailoring the enzyme towards a specific cellular task: the kinesin transducer element. From this work, we propose the chemomechanical outcomes of the motor domain evolve through modification of transducer behavior via sequence modifications of L5 and core beta-strand residues. This hypothesis broadens models that the cellular role of a kinesin evolves through duplication/motif addition or by domains outside the motor head. Second, our study reveals that motifs within the transducer have properties associated with NTPase switches and have family-specific sequence consensus. Thus, unique elements of the transducer system in NTPases may be exploited as targets for chemical inhibitors with inherently high selectivity.

1860-Pos Board B630
Probing the Structural and Energetic Basis of Kinesin-Microtubule Binding using Computational Alanine-Scanning Mutagenesis
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Kinesin-microtubule (MT) binding plays a critical role in facilitating and regulating the motor function of kinesins. To obtain a detailed structural and energetic picture of kinesin-MT binding, we have performed large-scale computational alanine-scanning mutagenesis based on long-time molecular dynamics (MD) simulations of kinesin-MT complex in both ADP and ATP state. First, we have built three all-atom kinesin-MT models for human conventional kinesin bound with ADP, and mouse KIF1A bound with ADP and ATP, respectively. Then, we have performed 30-ns MD simulations followed by kinesin-MT binding free energy calculations for both wild type and mutants obtained after substituting each kinesin charged residue by alanine. We have found that the kinesin-MT binding free energy is dominated by van der Waals interactions and further enhanced by electrostatic interactions. The calculated mutational changes in kinesin-MT binding free energy are in excellent agreement with an experimental alanine-scanning study with root mean squared error of ~0.32 kcal/mol). We have identified a set of important charged residues involved in the tuning of kinesin-MT binding, which are clustered on several secondary structural elements of kinesin (including well-studied loops L7, L8, L11, L12 and helices α4, α5, α6, and less-explored loop L2). In particular, we have found several key residues with different contribution to kinesin-MT binding in ADP and ATP state. The mutations of these residues are predicted to fine-tune the motility of kinesin by modulating the conformational transition between ADP state and ATP state of kinesin.

1861-Pos Board B631
Determining Molecular Motors Processivity
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In this work, we present two novel biochemial models describing the traslational motion of molecular motors such as kinesin or dynein. Both models allow to reconstruct the corresponding energy landscape of cycle catalyst process via molecular motors activity. The first one predicts no finite time for the processivity because lacks of inhibition reactions. The second model considers inhibition by ADP and predicts a finite time duration of the motion of the motor. Explicit expressions for the displacement velocity of the motors are also given.

1862-Pos Board B632
Role of Direct Motor-Motor Interactions on the Cooperativity and Microtubule Decoration by Ncd
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Ncd is a Kinesin-14 family motor protein. They walk cooperatively as a group to slide or crosslink microtubules (MT) in the mitotic spindle. Using structural analyses and coarse-grained molecular dynamics simulations we studied the longitudinal and lateral interactions between two neighboring Ncd dimers on a MT lattice. When bound on the same MT protofilament, we found that the leading (on the minus-end side) Ncd in the pre-stroke state prevents the lagging Ncd from making a step via steric hindrance between their neck coiled-coil domains. Such a longitudinal interaction may lead to synchronization in the motility cycle among Ncds bound along a single MT protofilament. Laterally, the nucleotide binding pocket (NBP) of a MT-bound head of a dimer on one protofilament is blocked by the surface loop L2 of the unbound head from an Ncd dimer on the neighboring protofilament. This occurs only when the latter Ncd is in the pre-stroke state. Since, this could lead to a temporary arrest of lateral Ncds in the pre-stroke state, it is likely that stepping would happen in a serial manner. This elucidates the potential role of the unbound MH of an Ncd dimer for lateral interactions between Ncds, which aids with the nucleotide-specific cooperative interaction.

1863-Pos Board B633
Structural Basis for the Regulation of Microtubule-Binding Affinity by Cytoplasmic Dynein
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The molecular motor cytoplasmic dynein is responsible for most minus-end directed microtubule-based transport in eukaryotic cells. Dynein is especially important in neurons, where defects in dynein-based motility have been linked to neurodegenerative and neurodevelopmental diseases in humans. Due to the complexity and size of dynein our understanding of it has lagged behind that of other cytoskeletal motor proteins. A major goal in the field is to determine how ATP hydrolysis events in dynein's AAA+ motor domain ''ring'' are communicated to the microtubule-binding domain (MTBD), which is located 250Å away. Information between these two sites is proposed to be conveyed via changes in the register of a long coiled-coil ''stalk'' that connects the MTBD to the AAA+ ring. Both low and high affinity registers have been identified and a crystal structure of the MTBD with the stalk locked into the low affinity register has been solved. To understand the structural changes that occur upon MT binding and how they might be regulated we have obtained a ~10Å resolution cryo-EM reconstruction of microtubules decorated with the MTBD of cytoplasmic dynein locked into the ''high affinity'' state. We used our density to perform molecular dynamics flexible fitting with the available crystal structure and observe significant rearrangements in three of the six alpha helices that make up the MTBD. Our pseudo-atomic model accounts for several mutations that were previously identified for their effects on microtubule-binding affinity. Based on our results we propose a model for the coordination between changes in the register of the coiled-coil and dynein's affinity for its track.

1864-Pos Board B634
The 2.8-Å Crystal Structure of the Dynein Motor Domain
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Dyneins are microtubule-based motor complexes that power a wide variety of biological processes within eukaryotic cells, including the beating of cilia and flagella, cell division, cell migration, and the intracellular trafficking. Most